REMARKS

Claims 1, 3-14 and 23-30 are now pending.

Support for the amendment to Claims 13 and 24 can be found, *inter alia*, in the description at page 7, lines 9-14; page 8, lines 28-32; and Claims 13, 21 and 22, as filed.

The amendment to Claim 29 to amend the term "test" to refer to "test sample", i.e., is being made to correct an obvious typographical error and is supported, for example, by Claims 1 and 25 from which Claim 29 depends.

The amendment to Claim 30 is being made to correct an obvious typographical error in the spelling of the word "amylase".

Accordingly, Applicants respectfully submit that the amendments to the claims do not constitute new matter, and thus request entry thereof.

In paragraph 3, on page 2 of the Office Action, the Examiner rejects Claims 14, 24, 29 and 30 under 35 U.S.C. § 112, second paragraph.

Specifically, the Examiner states that Claims 14 and 29 are confusing because it is unclear how a "quantity" of α-amylase in a test sample can be measured by comparing the detected binding of the sample to α-amylase "enzyme activities" in a standard or how it can be measured by comparing the detected binding of the sample to Falling Numbers. The Examiner contends that the activity of an enzyme is not necessarily related to its concentration, and that Falling Numbers are usually expressed in terms of viscosity of a sample and is normally a means to gauge

the level of preharvest sprout activity in cereal grains, such as wheat and barley.

For the following reasons, Applicants respectfully traverse the Examiner's rejection.

It is well-known to produce a standard curve between two parameters to thereby enable qualification of one parameter based on the value identified for another parameter. All that is required is a correlation between parameters.

The present application clearly exemplifies a correlation of the level of binding of an antibody to α-amylase in a grain sample (determined using an ELISA) with the level of viscosity of the same grain sample (determined using a Falling Number assay) (see page 22, line 6 to page 24, line 25). The standard curve produced using this method was then plotted as a graph shown in Figures 5A and 5B. Similarly, a standard curve was produced correlating the level of binding of an antibody to α-amylase in a grain sample (determined using an ELISA) with the level of α-amylase emzyme activity (determined using a Ceralpha assay) (see page 25, line 26 to page 26, line 9). By making such a comparison, the skilled person is capable of determining a Falling Number value or α-amylase activity level based on the level of antibody binding.

Both Falling Number values and α-amylase activity levels have previously been correlated with levels of α-amylase in a sample (see Physiochemical Tests, AACC Method 56-81B, "Determination of Falling Number", pages 1-4 (1999); and Enzymes, AACC Method 22-02, "Measurement of α-Amylase in Plant

and Microbial Materials Using the Ceralpha Method", pages 1-5 (2001), a copy of each of which is attached hereto).

Accordingly, Applicant respectfully submits that it would be apparent to the skilled person how the correlation between antibody binding to α-amylase and Falling Numbers or α-amylase enzyme activity may be used to quantify the level of α-amylase in a sample.

The Examiner also contends that Claim 24 is confusing because it is unclear how the aqueous extract comprises NaCl or $CaCl_2$, i.e., the claim appears to recite the test sample, itself comprises NaCl or $CaCl_2$ and not the aqueous extraction solution as disclosed by the specification.

Claim 13 has been amended to define the test sample as being an extract from grain, grain meal or flour in aqueous extraction medium, optionally comprising NaCl or $CaCl_2$. Applicant respectfully submits that the claims as amended make it clear that the aqueous extraction medium may comprise a specific salt, and not the test sample itself.

Accordingly, Applicant respectfully submits that the claims clearly and definitely recite the invention of interest, and thus request withdrawal of the Examiner's rejection.

On page 3 of the Office Action, the Examiner rejects Claims 25-29 under 35 U.S.C. § 112, first paragraph for failing to meet the written description requirement and for failing to be enabled by the specification.

The Examiner contends that the specification is enabling for determining the presence or amount of α-amylase in a sample, but does not provide enablement for a method for determining

weather damage in a plant or crop. The Examiner contends that the specification fails to positively correlate the presence of α-amylase to weather damage and that pre-harvest sprouting could be caused by contamination or the storage condition of grain silos that does not involve weather damage *per se*.

For the following reasons, Applicant respectfully traverses the Examiner's rejection.

It is not necessary for the specification to demonstrate that α-amylase in a cereal crop is associated with weather damage, as this was already well-known in the art as of the filing date of the application. For example, Meredith et al (*In*: Advances in Cereal Science and Technology, Volume VII, Americal Association of Cereal Chemists, St. Paul, MN, pages 239-320 (1985), a copy of which is submitted herewith) provide a comprehensive review of factors involved in pre-harvest sprouting (or weather damage). In fact, in the first paragraph of this chapter (at page 239) the authors state that the "chief culprit [in weather damage of cereal crops] is enzyme α-amylase..."

Meredith et al also describes a large number of assays that were in use at or prior to 1985 for determining weather damage in a crop (see at page 273 to page 284). Of these, the majority of methods detect the level of α-amylase in a cereal sample to detect weather damage, for example, the Falling Number method, the amylograph method and the penetrometer test. Several of

these methods have even become standard methods of the AACC, for example:

(i) AACC Method 22-08, "Measurement of α-amylase Activity with the Rapid-visco Analyzer" (November 8, 1995), a copy of which is attached hereto; and

(ii) AACC Method 22-10, "Measurement of α-amylase Activity with the Amylograph" (May 5, 1960), a copy of which is attached hereto.

As will be apparent from the foregoing, it was well established before the filing date of the instant application that a method for determining the level of α-amylase in a sample was useful for, for example, determining weather damage in a cereal grain or flour derived therefrom.

Of the methods described by Meredith et al, the most commonly used may be The Falling Number assay. This assay was developed to predict the level of weather damage (or sprout damage), as described at page 218, third paragraph of Hagberg, *Cereal Chem.*, 37:218-222 (1960), a copy of which is attached. Since its development, the Falling Number method has become a standard method for determining weather damage in cereals (as evidenced by its inclusion as AACC recognized method 56-81B (1999)). In fact, even the Examiner recognizes that the Falling Number method is used to determine pre-harvest sprouting in a cereal grain at paragraph 3 of the Office Action.

To confirm that efficacy of the inventive process for determining weather damage in cereal crops, the inventor compared the level of α-amylase detected using the two-site

immunoassay of the invention with the level of α-amylase detected using the Falling Number method in sprouted wheat (see, for example, page 22, line 6 to page 24, line 25 of the specification). As shown in Figures 5A and 5B, the results obtained with the two-site immunoassay of the invention correlated well with those determined using the Falling Number method.

Applicant respectfully submits that the art also recognizes α-amylase assay as a standard method for determining weather damage. For example, McCleary et al, *J. Cereal Sci.*, 6:237-251 (1987) (a copy of which is attached hereto), determined α-amylase levels using blocked p-nitrophenyl maltoheptaoside (BPNG7) (i.e., the Ceralpha method). For determining α-amylase levels in a cereal crop for determining the level of weather damage, the authors compared the level of α-amylase detected using the described method and the level of α-amylase detected using the Falling Number method. The authors also compared the level of α-amylase detected in flour that was spiked with a known level of purified α-amylase. Thus, α-amylase is a known predictor of weather damage. Since its initial development, the Ceralpha method has become a standard method for determining weather damage in a cereal crop and has become recognized by the AACC (AACC Method 22-02 (2001)). Applicant directs the Examiner's attention to the fact that the results attained using the two-site immunoassay of the invention correlated with those obtained using the Celalpha method using sprouted wheat (see page 25, line 26 to page 26, line 9 of the present specification).

Applicant respectfully submits that the demonstration that the immunoassay of the invention detects sprouted wheat as effectively as the Falling Number method or the Celephra assay clearly establishes that the method is effective at determining weather damage.

As for the Examiner's allegation that pre-harvest sprouting may be caused by factors other than weather damage, it is generally accepted in the art that environmental changes, in particular rain, cause pre-harvest sprouting (see, for example, Meredith et al (1985). Thus, an increase in α-amylase levels or activity is a measure of weather damage or sprouting damage in seed crops. This is evidenced by the large number of assays described herein and, for example, in Meredith et al (1985) that detect α-amylase to determine weather damage. On this basis, Applicant respectfully submits that it is not necessary to demonstrate that α-amylase is correlated with weather damage in a cereal crop.

The Examiner further contends that the specification does not provide any data correlations as to the amount of α-amylase detected and weather damage in a crop.

Applicants respectfully submits that the specification as filed clearly describes methods for producing a sample from a cereal for analysis (for example, at page 22, line 25 to page 23, line 9; and page 26, lines 13-15 of the present specification) in addition to methods for performing a two-site ELISA (for example, at page 17, line 14 to page 18, line 33; and page 22, line 6 to page 25, line 23 of the present specification) and a two-site immunochromotagraphy assay (for

example, at page 25, line 26 to page 27, line 22 of the present specification). These assays were shown to be capable of differentiating between unsprouted and sprouted wheat samples with results attained similar to those observed with the Falling Number method or Ceralpha method. On the basis of these results, Applicant respectfully submits that the instant application provides sufficient description to enable the skilled person to prepare and assay a sample from a cereal to determine the level of α-amylase in the sample and to determine whether or not the sample comprises pre-harvest sprouted (or weather damaged) cereal.

In fact an immunochromatography assay essentially as described in the instant application was used to assess weather damage in a variety of cereal crops through Australia. The results of this study was published in Skerritt et al, *Crop Sci.*, 40:742-756 (a copy of which is attached hereto). Using the immunochromatography assay or a Falling Number assay, the authors determined the level of α-amylase in an initial set of 14 grain samples from Australia with varying degrees of weather damage (e.g., with Falling Number between 100 s and 403 s) and 36 samples subjected to controlled wetting (with Falling Number between 85 s and 423 s). As discussed at page 746, right-hand column and shown in Figures 3A and 3B, the immunochromatography method produced results that closely correlated with the Falling Number results (linear regression r^2 = 0.954 or r^2 = 0.970, depending on the detection method used). The results attained using the immunochromatography method were also shown to be

reproducible with little within or between assay variation (as discussed at page 746, right-hand column thereof).

Skerrit et al also describe a collaborative trial of the immunochromatography assay in which 79 farmers assess the degree of weather damage in six samples that have varying degrees of weather damage as assessed using the Falling Number method. As discussed at pages 751 and 752, the majority of test subjects accurately estimated the Falling Number of each of the six samples using only the immunochromatography method. Furthermore, the majority of test subjects were able to determine the grade of the wheat tested (usually determined using a Falling Number test) based on the results of the immunochromatography test.

The immunochromatography assay used in Skerrit et al employs the methodology described in the instant application. This assay has been clearly shown to be useful for determining the level of weather damage in a cereal sample. Proceeding on this basis, Applicant submits that instant application clearly enables the production of an assay that determines weather damage in a cereal crop.

Accordingly, Applicants respectfully submit that the claims have written description support and are enabled by the present specification. Thus, Applicants request withdrawal of the Examiner's rejection.

On page 4 of the Office Action, the Examiner rejects Claims 1, 3-14, 23 and 25-30 under 35 U.S.C. § 102(b) as being anticipated by, or, in the alternative, under 35 U.S.C. § 103 as being patentable over Sander et al.

In addition, in paragraph 9, on page 5 of the Office Action, the Examiner rejects Claims 1, 3-14 and 23-30 under 35 U.S.C. § 102(b) as being anticipated by, or, in the alternative, under 35 U.S.C. § 103 as being obvious over LeCommandeur et al.

Specifically, the Examiner states that Sander et al and LeCommandeur et al disclose a two-site or sandwich enzyme-linked immunoadsorbent assay for α-amylase using monoclonal antibodies for binding distinct or two different epitopes of the α-amylase. The Examiner notes that Sanders et al and LeCommandeur et al are silent with respect to the epitopes of α-amylase to which the antibodies bind. However, the Examiner contends that since Sander et al and LeCommandeur et al disclose binding of the antibodies to distinct epitopes of α-amylase and SEQ ID NOs:1, 2 and 3 are inherently present in the α-amylase of the prior art, the claims are anticipated by or obvious over Sanders et al and LeCommandeur et al.

For the following reasons, Applicant respectfully traverses the Examiner's rejections.

Sander et al is merely directed to the development of a two-site immunoassay for determining the levels of fungal α-amylase, such as, for example, from *Aspergillus oryzae*, to determine the amount of fungal α-amylase in a test sample. The prior art assay is therefore useful for assessing the amount of a fungal allergen associated with a condition such as, for example, asthma, rhinitis, conjunctivitis and contact urticaria. These adverse conditions are particularly prevalent in bakers, as fungal amylases are added to flour in order to increase dough

volume and elasticity. Sander et al teach that their assay is intended to "establish dose-response relationships for the causative allergens and to define threshold limits below which no sensitization occurs" (page 99, left column).

The Examiner's allegation appears to be based on the belief that the specific epitopes to which the antibodies used in the method as claimed bind are an inherent feature of the *Aspergillus* enzyme.

However, the assay developed by Sander et al specifically recognizes fungal α-amylase, not α-amylase of wheat or rye at a useful level (see, for example, the paragraph bridging pages 97 and 98, page 88, right hand column and Figure 3B). This lack of cross-reactivity was considered to be an important feature of the assay described by Sander et al. In summary, because the assay described by Sander et al specifically recognizes fungal α-amylase as evidenced by lack of cross-reactivity with flour α-amylase, Sander et al, does not inherently disclose a single epitope of wheat α-amylase or an antibody that binds wheat α-amylase. Thus, Sander et al does not describe the essential features of the presently claimed invention.

As for Lecommandeur et al, such teaches the production of monoclonal antibodies that are capable of binding to barley α-amylase. α-amylase is a protein that comprises in excess of 400 amino acids (425 amino acids for wheat α-amylase and 437 for α-amylase). As would be apparent to the skilled artisan, a protein of this size comprises a large number of epitopes. In fact, the instant application shows that antibodies generated while producing the claimed immunoassay were capable of

recognizing a large number of epitopes distributed over the length of the amino acid sequences of wheat α-amylase (see, for example, Figure 3). However, an antibody raised against α-amylase may recognize any epitope shown in Figure 3 or any other epitope in the protein. Applicant submits that the ordinary skilled in the art would not have considered that the monoclonal antibodies produced by Lecommandeur et al would have necessarily recognized any of the epitopes recited in the claims rather than any other eiptope in α-amylase. As a consequence, Lecommandeur et al does not describe the essential features of the presently claimed invention.

Applicant also traverses the Examiner's allegation that the claimed are obvious in light of Sander et al or Lecommandeur et al. There is no actual disclose of any epitope of α-amylase in either document.

With regard to Lecommandeur et al, as discussed above, such merely discloses methods for production of monoclonal antibodies against barley α-amylase. This citation merely mentions that ELISA type assays may be useful in detecting α-amylase levels in wheat, but does not indicate how any specific epitope can be used to perform such a method. Thus, the disclosure is merely an invitation to experiment and does not render obvious a two-site immunoassay that comprises antibodies that recognize one or more specific epitopes.

With regard to Sander et al, Applicant submits that this citation would not lead the ordinary skilled artisan at the priority date to attempt to develop an assay detecting a plant α-amylase (i.e., an α-amylase comprising an epitope recited in

Claim 1), as any plant α-amylase protein in the samples described in the citation would contribute to background in that assay.

Furthermore, Sander et al addresses a problem that is conceptually entirely different to the problem addressed by Lecomandeur et al or the present invention. The skilled artisan would not look to Sander et al when attempting to develop an immunoassay for assessing weather damage of a crop plant, pre-harvest sprouting, or even the detection of plant α-amylases.

Applicant also respectfully reminds the Examiner that the claimed invention in not merely any two-site immunoassay capable of detecting any α-amylase, but an immunoassay that requires antibodies that bind to specific epitopes in an α-amylase enzyme as set forth in SEQ ID NOs:1-3. As discussed above these epitopes are not disclosed in either citation. As a consequence, the claims on file are limited to a novel and inventive class of antibodies that bind to the epitopes set forth in SEQ ID NOs:1-3.

It would have been necessary for the skilled artisan to exercise inventive effort to produce the novel and inventive class of antibodies used in the immunoassay as presently claimed. This is because: (i) the degree of homology between *Aspergillus* and wheat α-amylase is too low to motivate a non-inventive skilled artisan to even attempt to identify the eiptopes recited in Claim 1 of the instant application; and (ii) the epitopes recited in Claim 1 of the application represent a selection of epitopes that were specifically useful

for two-site immunoassays, since not all epitopes against α-amylase actually work in a two-site immunoassay (see page 19, lines 3 to 9, of the specification).

The disclosure at page 19, lines 3 to 9, of the present specification makes it abundantly clear that not all antibodies raised against α-amylase are capable of detecting α-amylase in a sandwich (i.e., two-site) ELISA. Antibodies that were not useful in a sandwich ELISA were found to bind to different eiptiopes to those defined in the claims (see page 20, lines 30 and 31 and Figure 3 of the present specification).

Moreover, the antibodies were ultimately determined to be useful in a sandwich ELISA were not those raised against the most immunodominant epitope(s) of α-amylase. As shown in Figure 1, monoclonal antibody 15724 recognized epitopes that were considerably more immunodominant that those defined in the claims. In contrast to the teaching provided in the specification, a non-inventive skilled artisan at the priority date would have expected monoclonal antibody 15724, against the immunodominant epitope of α-amylase, would not been most suitable for any immunoassay that detects α-amylase in a test sample. Counterintuitive to conventional wisdom in the art at the priority date, the Applicant found that antibodies against the immunodominant epitope were not necessarily suitable for use in a two-site immunoassay and, in particular, an assay capable of being performed in the field. Applicant found that the antibodies that recognized at least one of three specific epitopes (as defined in Claim 1), that were not as

immunodominant as those recognized by monoclonal antibody 15724, were more effective in a two-site assay.

Accordingly, Applicants respectfully submit that the present invention is not taught or suggested in Sander et al or LeCommandeur et al, and thus request withdrawal of the Examiner's rejection.

In view of the amendments to the claims and the arguments set forth above, reexamination, reconsideration and allowance are respectfully requested.

The Examiner is invited to contact the undersigned at his Washington telephone number on any questions which might arise.

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